

Client's Ref.:

File: 0470-5039-US Final Frank

WHAT IS CLAIMED IS:

- 1 1. A method for cultivation of filamentous fungi
2 comprising the steps of:
3 (a) preparing a medium comprising a suspended
4 nutritionally solid substrate; and
5 (b) inoculating an inoculum into said medium comprising
6 said nutritionally solid substrate in a bioreactor to carry
7 out fermentation.

- 1 2. The method as claimed in claim 1, wherein said
2 filamentous fungi comprise *Monascus*, *Penicillium* or
3 *Aspergillus*.

- ~~Sub C2~~ 3. The method as claimed in claim 1, wherein said
2 nutritionally solid substrate is a carbohydrate.

- 3 4. The method as claimed in claim 3, wherein said
4 carbohydrate is grain.

- 1 5. The method as claimed in claim 4, further comprising
2 the steps of husking, cocking and sterilizing said grain
3 before adding to said medium.

- 1 6. The method as claimed in claim 1, wherein said
2 medium in step (a) further comprises a nitrogen source,
3 inorganic salts and trace elements.

- 1 7. The method as claimed in claim 1, further comprising
2 a step of inoculating said filamentous fungi after step (a)
3 to obtain said inoculum, and then inoculating said inoculum

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4 into said medium comprising said nutritionally solid
5 substrate in a bioreactor to carry out fermentation.

1 8. The method as claimed in claim 7, wherein the step
2 of inoculating said filamentous fungi comprises:

3 (1) inoculating said filamentous fungi from a stock
4 culture to a new agar plate and incubating in an incubator
5 for 5 ~ 7 days;

6 (2) washing spores and mycelia grown on said plate with
7 sterile water;

8 (3) cultivating said spores/mycelia in a medium
9 comprising a nutritionally solid substrate by shaking; and

10 (4) inoculating a culture cultivated for 36 ~ 48 hours
11 at step (3) into a bioreactor.

1 Sub 107 9. The method as claimed in claim 8, wherein said
2 bioreactor is a pneumatic bioreactor.

1 10. The method as claimed in claim 9, wherein said
2 pneumatic bioreactor is an air-lift bioreactor with a net
3 draft tube.

1 11. The method as claimed in claim 1, further
2 comprising cultivating said filamentous fungi using the fed-
3 batch process.

Sub 104 12. The method as claimed in claim 11, wherein the
2 medium of the batch comprises a nitrogen source and a
3 nutritionally solid substrate.

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1 13. A method for cultivation of *Monascus* species by
2 using a suspended grain substrate comprising the steps of:

3 (a) preparing a medium comprising a suspended grain
4 substrate; and

5 (b) inoculating an inoculum into said medium comprising
6 said grain substrate in a bioreactor to carry out
7 fermentation.

1 14. The method as claimed in claim 13, further
2 comprising the steps of husking, cocking and sterilizing
3 said grain before adding to said medium.

1 15. The method as claimed in claim 13, further
2 comprising a step of inoculating said *Monascus* species after
3 step (a) to obtain said inoculum, and then inoculating said
4 inoculum into said medium comprising said nutritionally
5 solid substrate in a bioreactor to carry out fermentation.

1 16. The method as claimed in claim 15, wherein the step
2 of inoculating said *Monascus* species comprises:

3 (1) inoculating said *Monascus* species from a stock
4 culture to a new agar plate and incubating in an incubator
5 for 5 ~ 7 days;

6 (2) washing spores and mycelia grown on said plate with
7 sterile water;

8 (3) cultivating said spores/mycelia in a medium
9 comprising a grain substrate by shaking; and

10 (4) inoculating a culture cultivated for 36 ~ 48 hours
11 at step (3) into a bioreactor.

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1 17. The method as claimed in claim 16, wherein said
2 bioreactor is a pneumatic bioreactor.

1 18. The method as claimed in claim 17, wherein said
2 pneumatic bioreactor is an air-lift bioreactor with a net
3 draft tube.

1 19. The method as claimed in claim 13, further
2 comprising cultivating said *Monascus* species using the fed-
3 batch process.

Sub 15
1 20. The method as claimed in claim 19, wherein the
2 medium of the batch comprises a nitrogen source and a grain
3 substrate.

Sub 15
1 21. A method for producing metabolites from the
2 cultivation of *Monascus* species by using a suspended grain
3 substrate comprising the steps of:

4 (a) preparing a medium comprising a suspended grain
5 substrate; and

6 (b) inoculating an inoculum into said medium comprising
7 said grain substrate in a bioreactor to carry out
8 fermentation.

Sub 15
1 22. The method as claimed in claim 21, further
2 comprising the steps of husking, cocking and sterilizing
3 said grain before adding to said medium.

Sub 15
1 23. The method as claimed in claim 21, further
2 comprising a step of inoculating said *Monascus* species after

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3 step (a) to obtain said inoculum, and then inoculating said
4 inoculum into said medium comprising said nutritionally
5 solid substrate in a bioreactor to carry out fermentation.

1 24. The method as claimed in claim 23, wherein the step
2 of inoculating said *Monascus* species comprises:

3 (1) inoculating said *Monascus* species from a stock
4 culture to a new agar plate and incubating in an incubator
5 for 5 ~ 7 days;

6 (2) washing spores and mycelia grown on said plate with
7 sterile water;

8 (3) cultivating said spores/mycelia in a medium
9 comprising a grain substrate by shaking; and

10 (4) inoculating a culture cultivated for 36 ~ 48 hours
11 at step (3) into a bioreactor.

1 25. The method as claimed in claim 24, wherein said
2 bioreactor is a pneumatic bioreactor.

1 26. The method as claimed in claim 25, wherein said
2 pneumatic bioreactor is an air-lift bioreactor with a net
3 draft tube.

1 sub 2147 27. The method as claimed in claim 20, further
2 comprising cultivating said *Monascus* species using the fed-
3 batch process.

2 28. The method as claimed in claim 27, wherein the
2 medium of the batch comprises a nitrogen source and a grain
3 substrate.